REMARKS

Per the Office Action dated September 15, 2009, prosecution of claims 66, 67, 87-94, and 133-141 was reopened pursuant to the Panel Decision from the Pre-Appeal Brief conference. The previous rejections under 35 U.S.C. § 102(e) and 35 U.S.C. § 103(a) were withdrawn. However, new rejections were made and all pending claims stand rejected.

Before addressing the instant rejections, Applicant would like to reiterate several key features of the claimed invention. The claimed invention comprises a micelle comprising a therapeutic bioactive component and hydrophobic surfactant, with a surrounding precipitate shell comprising a polypeptide ligand and a precipitating cation, that results in a nanocapsule of less than about 50 nanometers in diameter capable of receptor mediated targeting and uptake into the cell. The claimed invention provides the first mechanically-stabilized sub-50 nm targeted particle encapsulating a therapeutic bioactive component, a particle that the prior art lacked and a particle that provides unexpected benefits.

The ultrasmall size of this unique composition is enabled in part by the core being formed by a transiently stable hydrophobic micelle; stabilization and targeting are efficiently provided by the precipitate polypeptide shell. The problems that are simultaneously addressed by the claimed invention include, for example, delivery of a therapeutic cargo intact into the cell by protecting the cargo from enzymatic degradation and by avoiding lysosomal degradation, in a cell targeted manner. Importantly, avoiding lysosomal degradation has been recognized in the art as a major obstacle to new, important therapies (see, for example, Varga et al.: lysosomal degradation is "believed to be the greatest barrier for successful gene expression in non-viral delivery vehicles."[1]) All publications discussed herein are attached to an IDS submitted together with this instant response, under separate cover.

DOUBLE PATENTING

The Examiner has provisionally rejected the claims 66, 67, 87, 88, 90, 94, 134 and 136-141 on the ground of nonstatutory obviousness-type double patenting as being

[1] C.M. Varga, et al. "Receptor Mediated Targeting of Gene Delivery Vectors: Insights from Molecular Mechanisms for Improved Vehicle Design", *Biotechnology and Bioengineering*: 70(6) 593-605 (2000).

unpatentable over claims 25-28 of copending Application No. 11/622,359. Office Action page 4.

The Examiner has provisionally rejected the claims 67, 87, 88, 90, 94, 133, 134 and 136-141 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 8 and 11 of copending Application No. 12/027,863.

The Examiner has also provisionally rejected the claims 66, 67, 87, 88, 90, 94, 133, 134, and 136-141 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 25-28 of copending Application No. 11/622,359.

The Examiner has also rejected the claims 66, 67, 87, 88, 90, 93, 94, 133, 134, and 136-141 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 29, 31, 33, 37, and 42 of U.S. Patent No. 6,632,671.

Applicant acknowledges these rejections and wishes to advise that Applicant will address the Examiner's double patenting rejections once all other rejections have been resolved, or earlier.

CLAIM REJECTIONS UNDER 35 U.S.C. § 103(a)

A. Rejection of claims 66, 67, 87, 88, 90-94, and 135-139

The Examiner has rejected claims 66, 67, 87, 88, 90-94, and 135-139, under 35 U.S.C. § 103(a) as being unpatentable over Unger, E.C. et al. (U.S. Patent No. 6,139,819, the "Unger et al" patent) in view of each Kondo et al., in view of each Medina (U.S. Patent No. 5,650,543), Quay (U.S. Patent No. 5,707,606) and Duquemin et al. (J Pharm Pharmacol, 1985, 37:698-702, Abstract.)

The Examiner contends that Unger et al. teach particles comprising a core provided by monomolecular layers of surfactant micelles consisting of a surfactant and a bioactive agent which has a therapeutic effect, wherein the surfactant micelles are stabilized by a surrounding protein shell; the protein shell is covalently coupled with targeting ligands that bind cell surface receptors (i.e., the protein provides specific cellular uptake), wherein the covalent coupling involves the formation of Schiff base linkages which are reduced by using lithium aluminum hydride. The Examiner further contends that the bioactive agent could be a polynucleic acid which is associated with cationic lipids (i.e., a condensing agent). The Examiner also contends that Unger et al. teach that the particles have a size of

about 30 nm and a hollow core comprising the bioactive agent, the particles can be in the form of nanocapsules, the particles can comprise a combination of two or more surfactants, a biocompatible oil, a water miscible solvent, and a polynucleic acid condensing agent. The Examiner also contends Unger et al.'s particles inherently possess a lithium-precipitated protein shell, since the covalent attachment of the targeting ligand requires addition of lithium aluminum hydride, which would necessarily result in a precipitated protein shell, and notes that lithium is known in the art as a protein precipitating agent, citing for example Kondo et al.

The Examiner contends that Unger et al. fails to teach that the surfactant is an acetylenic diol such as 2,4,7,9-tetramethyl-5-decyne-4,7-diol, i.e. surfactants with an HLB of less than about 6; but Medina suggests these diols and their ethoxylates as excellent surfactants because of their ability to decrease the surface tension. Quay teaches acetylenic diols or blends thereof for the prep of stable and biocompatible nanoparticles since these diols lower surface tension. Duquemin et al. teach that reducing surface tension results in smaller particles. Accordingly it would have been obvious, according to the Examiner, to modify the particles of Unger by using 2,4,7,9-tetramethyl-5-decyne-4,7-diol because the art teaches use of acetylenic diols results in nanoparticles with improved properties.

Applicant traverses this rejection, on several grounds.

1. *Lack of teaching-suggestion-motivation from the prior art.*

KSR affirmed the long standing principle that a "patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." (*KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 298, 418 (2007)). As discussed in the MPEP at Section 2141, the Supreme Court recognized the teaching-suggestion-motivation (TSM) test as one of a number of valid rationales that could be used to determine obviousness. *KSR International Co. v. Teleflex, Inc.,* 550 U.S. 398 (2007). Legally it is also required that in addition to a finding of TSM, there must also be a finding that there was a "reasonable expectation of success." See MPEP § 2141.02(g).

The Examiner appears to be using TSM as a basis for the rejection. Specifically, the Examiner states that the combination of the references suggests the use of nanoparticles with surfactants with an HLB of less than about 5 for the purpose of "improved properties."

It is submitted that must be a motivation to combine the references in the same way as Applicant, either explicit in the art or known to the ordinary skilled person. The Examiner states that the ordinary skilled person, at the time the invention was made, would have been motivated to combine the references cited to create nanoparticles with "improved properties." As explained below, that there is no reason why a skilled person would seek to make sub-50 nm particles, and/or particles with precipitated shells (i.e., more rigid particles), based on knowledge in the art, or based on the express teachings of the references. Further, there is no expectation of success.

a. *Motivation*. It is submitted that the Examiner has not provided the "apparent reason to combine the known elements in the way the patent claims":

> To determine whether there was an apparent reason to combine the known elements in the way a patent claims, it will often be necessary to look to interrelated teachings of multiple patents; to the effects of demands known to the design community or present in the marketplace; and to the background knowledge possessed by a person having ordinary skill in the art. To facilitate review, this analysis should be made explicit." *KSR* (emphasis added).

Applicant disputes the Examiner's finding of motivation. First, the Examiner states that the art teaches use of acetylenic diols to result in nanoparticles with improved properties. Applicant submits that the particles of the instant invention have, as one aspect, their small size. Another aspect of the inventive particles is the precipitated nature of the shell. There was no reason for skilled artisans to pursue the claimed particles with their properties such as being small and also being precipitated, based on the references' teachings, as explained further below. The Examiner has not identified any design need or market pressure to solve a problem, whether or not disclosed by Unger et al, that would be optimally addressed with sub-50 nanometer, rigid particles, much less the sub-50 nanometer, rigid nanocapsules of the claimed invention. This point is explained more fully below.

The Examiner's failure to provide evidence supporting the rationale for an artisan of ordinary skill to pursue the described combination is a clear error in view of the MPEP and *KSR*.

Applicant submits the rationale for creating the claimed nanocapsules are simply not taught or suggested in Unger et al. Specifically, the claimed invention is directed toward a surfactant micelle comprising a therapeutic bioactive component and a hydrophobic surfactant, surrounded by a precipitate shell comprised of a polypeptide and a cationic precipitating agent, which provides cell-targeted delivery and uptake via receptor mediation, in a capsule measuring less than about 50 nanometers in diameter. Claim 138 specifies that the cationic precipitating agent is Li+. In contrast, Unger et al. teaches 200 nm-to micron-sized formulations of inert gas or gaseous precursors encapsulated in flexible lipids for use in conjunction with ultrasound that can visualize or rupture said formulation at a targeted extracellular site. This characterization is supported for example by the following disclosures in Unger et al.

> [T]here is a need for improved ultrasound techniques, including improved contrast agents which are capable of providing medically useful images…. (Col 3, lines 66-67, and Col.4, lines 1-2) ¶ [B]ubbles, including gas-filled bubbles, are useful as contrast agents. The term "bubbles", as used herein, refers to vesicles which are generally characterized by the presence of one or more membranes or walls surrounding an internal void that is filled with a gas or precursor thereto. Exemplary bubbles include, for example, liposomes, micelles and the like. As discussed more fully hereinafter, the effectiveness of bubbles as contrast agents depends upon various factors, including, for example, the size and/or elasticity of the bubble. ¶ With respect to the effect of bubble size, the following discussion is provided. As known to the skilled artisan, the signal which is reflected off of a bubble is a function of the radius (r.sup.6) of the bubble (Rayleigh Scatterer). Thus, in the frequency range of diagnostic ultrasound, a bubble having a diameter of 4 micrometer (.mu.m) possesses about 64 times the scattering ability of a bubble having a diameter of 2 μm. Thus, generally speaking, the larger the bubble, the greater the reflected signal. ¶ However, bubble size is limited by the diameter of capillaries through which the bubbles must pass. Generally, contrast agents which comprise bubbles having a diameter of greater than 10 μm can be dangerous since microvessels may be occluded. Accordingly, it is desired that greater than about 99% of the bubbles in a contrast agent have a diameter of less than 10 μm. **Mean bubble diameter is important also, and should be greater than 1 μm, with greater than 2 μm being preferred.** The volume weighted mean diameter of the bubbles should be about 7 to 10 micrometer. ¶ **The elasticity of bubbles is also important. This is because highly elastic bubbles can deform, as necessary, to "squeeze" though capillaries and/or to permit the flow of blood around the bubbles. This decreases the likelihood of occlusion.** (Col. 4, lines 6-41)… ¶ Accordingly, new and/or better contrast agents and methods for

providing same are needed. The present invention is directed to this, as well as other important ends. (Col 5, line 56-58) (emphasis added)

Applicant's characterization of Unger et al's teaching as a whole is further supported by the reference's preferred elements and embodiments, as reflected in the specification:

Unger et al preference (emphases added)	Source
5. It is "especially preferred" that the internal void be filled 100% with a gas or gaseous precursor	Col 8, lines 42-43
6. "The gas provides the lipid-based compositions with enhanced reflectivity, particularly in connection with vesicle compositions in which the gas is entrapped within the vesicles. This may increase their effectiveness as contrast agents."	Col 23, lines 38-43
7. Preferred gases are those which are inert and biocompatible, with perfluorocarbons being both preferred gases and gaseous precursors as well as a preferred stabilizing compound.	Col 23, lines 44-45, Col 26, lines 31-32, Col. 27, lines 44-52, Col. 29, lines 56-58
8. Most preferred "vesicle" size is 200 nm to about 7 um ("Vesicles" are broadly defined as "spherical entities comprising one or more walls or more membranes which form one or more internal voids" and include "liposomes, micelles, bubbles, microbubbles, microspheres, lipid-, polymer- protein- and/or surfactant-coated bubbles, microbubbles and/or microspheres, microballoons, aerogels, clathrate bound vesicles, and the like".)	Col. 7, lines 28-59, Col. 28, line 57
9. In preferred embodiments, targeted compounds are incorporated in compositions which are used to form targeted vesicles, and changes, for example, in pH and/or temperature in vivo, may be employed to promote a change in location in the targeting ligands, for example, from a location within the vesicle, to a location external to the outer wall of the vesicle, or that high energy ultrasound can also be used to rupture the vesicle to expose the targeting ligand to the binding site.	Col. 53, lines 42-51 and Col. 54, lines 32-44
10. Compositions of the present invention are particularly useful in connection with ultrasound, including diagnostic and therapeutic ultrasound, and that the use of the compositions with ultrasound is "described throughout the present disclosure".	Col. 55, lines 1-5
11. For example, ultrasound may be used to visualize the vesicles and verify the localization of the vesicles in certain tissue. In addition, ultrasound may be used to promote rupture of the vesicles once the vesicles reach the intended target, including tissue and/or receptor destination, thus releasing a bioactive agent and/or diagnostic agent.	Col. 81, lines 64-67, and Col 82, lines 1-2
12. As a preferred embodiment of the invention, gas filled vesicles are targeted to atherosclerotic plaque to noninvasively detect diseased blood vessels before damage has occurred.	Col. 36, lines 64-67

Two important characteristics of the Unger particles is that (1) their **"[m]ean bubble diameter . . . should be greater than 1 µm, with greater than 2 µm being preferred. The volume weighted mean diameter of the bubbles should be about 7 to 10 micrometer**." (Col. 4, lines 6-41) In other words, for optimal efficacy for the Unger

invention, that is, particles for ultrasound imaging, the particles are taught to be significantly larger than the Applicant's particles, i.e., **greater than 2 µm, whereas Applicant's particles are less than 50 nm**. The Unger size preference is to cause the bubbles to be detectable by ultrasound analysis. There is a fourfold difference between the references' recommended size and Applicant's size particles. There is no teaching in Unger that a significantly smaller size particle could have any benefit; in fact, such small particles are characterized as "not preferred," because the smaller the bubble, the more difficult to visualize by ultrasound. Therefore there is no motivation to make the Unger bubbles smaller.

The other important characteristic taught by Unger as seen by the excerpt provided, above, is (2) "[t]he elasticity of bubbles is also important. This is because highly elastic bubbles can deform, as necessary, to 'squeeze' though capillaries and/or to permit the flow of blood around the bubbles. This decreases the likelihood of occlusion." (Col. 4, lines 6-41) Highly elastic bubbles are taught by Unger to allow penetration of the bubbles into capillaries. In contrast to the teachings of Unger's highly elastic bubbles, the Applicant's particles are more rigid. Applicant has increased the rigidity of Applicant's particles by cationic precipitation of a polypeptide shell, by Li^+ for example. Thus, Applicant's teaching of increasing the rigidity of a particle (i.e., by cationic precipitation of a polypeptide shell) is another example of why the person of skill in the art would not have been led to modifying the Unger particles as to make them less flexible and more rigid, because according to Unger, flexibility of the bubbles is important.

The secondary references do not provide the missing motivation. Duquemin is cited for teaching the desirability of a "smaller coacervate droplets," but Duquemin is concerned with establishing the relationship between a particular surfactant (SLS) and droplet size, and does not establish the desirability of a small size nor does Duquemin provide any quantitation of what "smaller" actually means. Quay, like Unger, is concerned with providing ultrasound contrast agents, comprising bubbles (air-entrapped) vesicles. The main problem that Quay is concerned with solving is the problem of stability of the bubbles (see Col. 2, lines 31-57, particularly, lines 51-57, where prior art emulsions are taught to be "unworkable as a commercial product" due to their short "shelf life," i.e., instability.) Quay teaches solutions to instability that includes manipulating HLB, NOT

small size or nor rigidity. Although Applicant has taught an HLB of less than about 6.0, Quay teaches surfactants with HLB's ranging from 10 to 19 yield the interfacial tension (IFT) (dynes/cm) necessary to obtain stability (see Example 13); as described further below, this finding certainly does not draw the skilled artisan toward the claimed nanocapsules, comprising a surfactant with HLB of less than about 6.0. The particle sizes taught in Quay are similar to the sizes taught by Unger, for the reason that ultrasound contrast agents have a desired size range, which is much greater than 50 nm. Medina suggests TM diols and their ethoxylates as excellent surfactants because of their ability to decrease the surface tension, but Applicant did not employ the Quay technique of decreasing surface tension in order to result in a more stable particle.

With particular regard to Quay, Applicant submits the Examiner has mischaracterized the reference in asserting that "Quay teaches the use of acetylenic diols or blends thereof for the preparation of stable and biocompatible nanoparticles, wherein acetylenic diols stabilize the nanoparticle by lowering the surface tension (column 3, lines 15-20, column 7, lines 9-16)." (Office Action, Page 12) Quay **does not** teach the use of low-HLB acetylenic diols (i.e., surfactants with HLB's of less than about 6.0) to produce stable nanoparticles.

Applicant submits Quay's teaching of acetylenic diols that reduce surface tension *and* stabilize the colloidal dispersion, is directed toward and limited to those surfactants, including acetylenic diols, that lower the interfacial tension (IFT) between the dispersed liquid and water to *below 26 dynes/cm*. (column 3, lines 15-20) This disclosure is further elaborated in Example 13, which states that the selection of "amphiphilic materials with the proper HLB number for the selected dispersed phase is important for the stability of the colloidal dispersion." (column 17, lines 37-39) Inspection of the table furnished in Example 13 reveals a U-shaped correlation between HLB number and IFT dynes/cm; an HLB of 22 produces an IFT above 26 dynes/cm (i.e., 26.36), while HLB's between 10-19 produce IFT's below 26 dynes/cm (with the nadir being a surfactant with HLB of 14 producing an IFT of 22.48 dynes/cm), and while an HLB of less than 10 produces an IFT above 26 dynes/cm (specifically, an HLB of 8 produces an IFT of 27.07). As Quay concludes, "the use of amphiphilic materials, such as anionic, nonionic, cationic, or zwitterionic surfactants with an HLB number of 14 will provide the greatest stability for

emulsions of the above liquid dispersed phase." (column 17, line 66 – column 18, line 4) Thus, upon reading Quay, one skilled in the art would understand that to produce stable compositions with amphiphilic surfactants such as acetylenic diols, the HLB of the surfactant must be between 10-19, with an HLB of 14 being the most preferred.

Applicant submits Quay in fact teaches away from the use of low-HLB acetylenic diols (i.e., surfactants with HLB's of less than about 6.0) (as is taught by Applicant), by presenting the U-shaped data in Example 13, showing that the greatest particle stability is achieved with components with HLB's between 10-19. Applicant submits the teachings of Unger et al, Medina, and Duquemin do not contradict Quay's teaching regarding HLB and particle stability.

Thus, Applicant submits that there is no motivation for the ordinary skilled person to combine the references, because the references do not motivate the ordinary skilled person to create achieve small, more rigid inventive particles based on the fact that Unger teaches a preferred size of 2 μm (much larger than Applicant's 50 nm or less) and a preference for a highly elastic bubble (in contrast to Applicant's precipitated shell, causing rigidity); Duquemin provides no motivation to make small sizes, and Quay, like Unger, teaches the conventional ultrasound contrast vesicles with a size range of greater than 50 nm and further recommends use of surfactants with an HLB of between 10-19, rather than the Applicant's 6 or less. These references do not provide the skilled person with any motivation to attempt a more rigid, small size particle, with low HLB surfactants, as rigidity and small size is undesirable for an ultrasound contrast agent.

Therefore, the skilled person has no motivation to create the particles taught by Applicant based on the teachings of Unger, nor of Unger combined with Kondo et al., in view of each Medina, Quay and Duquemin et al. Reconsideration is respectfully requested.

b. *reasonable expectation of success*. Legally it is also required that in addition to a finding of TSM, there must also be a finding that there was a "reasonable expectation of success." See MPEP § 2141.02(g).

Heidel et al. summarized the views held by many for years, i.e., that addressing the challenges of drug delivery is not simply a matter of identifying useful ingredients or adding components to known vesicle structures; rather, the prevailing view is that"the

challenges of creating a nonviral delivery system are many[, but] we believe that one of the most daunting….is the integration of the components into a workable system that combines the attributes of the components without suffering losses because of their integration."[2]

Applicant submits the references do not disclose the parameters for or the results of obtaining a particle similar to particular form of the claimed nanocapsules, and therefore one skilled in the art, armed with the knowledge of the tremendous and well known challenges of integrating components into functional nanoparticles, and would not have had a reasonable expectation of success based on the references cited.

The state of the art at the time viewed targeting vehicle binding to a specific cell receptor for subsequent endosomal uptake into the cell as an attractive drug delivery strategy. Nonetheless, with respect to gene delivery, for example, a contemporaneous review article observes that while this:

> "seemingly simple concept has been pursued for more than a decade…in practice, this idea has been more difficult to implement effectively than perhaps had been originally anticipated….Effective gene delivery by [the] receptor mediated mechanism requires specific vector binding, internalization, subcellular trafficking, endosomal escape, and unpackaging of the foreign DNA for desired gene expression. While this process offers a noninvasive mechanism to obtain selective intracellular localization of vectors, it may lead to destruction of delivered genes through intracellular pathways…"[3]

Thus at the time of the instant invention, there is evidence to show that one skilled in the art would have been doubtful of the probability of success, for developing an integrated delivery system designed for cell-specific targeting and uptake.

It is further submitted that it was known at the time of the instant invention, that the manufacture of a liposome of less than 100 nanometers was difficult to achieve due to their intrinsic instability. See, e.g., Kong et al., (cosubmitted in an IDS.) Specifically, in a study of tumor-specific liposomes of a range of sizes, "[l]iposomes < 100 nm in diameter were not made because of instability." G. Kong et al., Hypothermia Enables Tumor-specific Nanoparticle Delivery: Effect of Particle Size, *Cancer Research*: 60, 4440-4445 (2000).

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[2] J. Heidel et al., "Molecular Conjugates", *Adv. Biochem. Engin./Biotechnol*: 99, 7-39 (2005). Copy provided in IDS accompanying this response.

[3] C.M. Varga, et al. "Receptor Mediated Targeting of Gene Delivery Vectors: Insights from Molecular Mechanisms for Improved Vehicle Design", *Biotechnology and Bioengineering*: 70(6) 593-605 (2000).

In another example described further below in this response, Schneider et al found that when polypeptide-DNA complexes were associated with a delivery vehicle (LipofectAMINE ^{TM}), targeting specificity was reduced, leading to the conclusion that "in the presence of the LipofectAMINE ^{TM} internalization of the complex occurs to a large extent by an integrin-independent mechanism." Again, this is another example of the significant challenges of integrating components into a viable drug delivery system.

Accordingly, based on objective evidence, , there was at the time of the instant invention no "expectation of success" for stable sub 50 nm particles with cell-targeting and uptake capabilities. The cited references provide no parameters or results to suggest otherwise. Thus, the Examiner must conclude that there is no expectation of success for the instant invention.

2. *USPTO guidelines for post-KSR consideration of obviousness* (October 10, 2007) MPEP §2141. The USPTO issued new examination guidelines that directed examiners to consider at least seven exemplary rationales that may support a conclusion of obviousness, including:(A) Combining prior art elements according to known methods to yield predictable results; (B) Simple substitution of one known element for another to obtain predictable results; (C) Use of known technique to improve similar devices (methods, or products) in the same way; (D) Applying a known technique to a known device (method, or product) ready for improvement to yield predictable results; (E) "Obvious to try" - choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success; (F) Known work in one field of endeavor may prompt variations of it for use in either the same field or a different one based on design incentives or other market forces if the variations are predictable to one of ordinary skill in the art; (G) Some teaching, suggestion, or motivation in the prior art that would have led one of ordinary skill to modify the prior art reference or to combine prior art reference teachings to arrive at the claimed invention.

Specifically, Applicant below argues in particular that the instant application is not "'Obvious to try' - choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success."

In the pharmaceutical arts, the Court stated that "[t]o the extent an art is unpredictable, as the chemical arts often are, *KSR*'s focus [on a finite set of] 'identified, predictable solutions' may present a difficult hurdle [for Examiners] because potential solutions are less likely to be genuinely predictable." *Eisai Co. v. Dr. Reddy's Labs., Ltd.*, 533 F.3d 1353 (Fed. Cir. 2008), reh'g denied, 2008.

It is submitted that the instant case is directed to a pharmaceutical art (making of liposome systems), and is thus in an area that is less likely to be predictable. Thus, the *KSR*'s standard for obviousness calling for analysis of the invention as a combination from a "finite number of identified, predictable solutions" is less likely to be applicable, as the instant invention is less likely to be predictable.

The liposomal arts are known to have significant technical challenges, and thus are not "predictable." Specifically, the art knowledge is that combining elements to yield workable liposome systems is challenging:

> the challenges of creating a nonviral delivery system are many[, but] we believe that one of the most daunting….is the integration of the components into a workable system that combines the attributes of the components without suffering losses because of their integration.[4]

It is clear, then, that the prior art teaches that the selection of components and creation of a workable system is viewed as a significant hurdle. Therefore, creating a liposome system is not simply a matter of using known components to produce a predictable solution.

Further, it is submitted that *KSR* requires that there be a "finite" number of "identified, predictable solutions." In contrast, the combination of the references provides an almost **infinite** number of heterogeneous solutions. Additionally, combining such heterogeneous solutions in the field of making liposome systems, as pointed out above, is not predictable. Applicant respectfully submits that the references' disclosures contains so many components and contains so many variations, that it cannot be concluded that there is a "finite number of identified, predictable solutions."

For example, Unger discloses a size range for "vesicles" spanning several orders of magnitude of between 30 nanometers and about 100 micrometers (Unger col. 28, lines 51-

[4] J. Heidel et al., "Molecular Conjugates", *Adv. Biochem. Engin./Biotechnol*: 99, 7-39 (2005). Copy provided in IDS accompanying this response.

53) with no guidance of where, in this vast range, one would begin to formulate the size of a "vesicle" if one sought to deliver a therapeutic agent into target cells in a non-degradative manner. Unger teaches that preferred vesicles are about 2 μm, much larger than Applicant's particles, as discussed above. Vesicles taught by Quay are similarly larger in size than Applicant's.

With regard to size, Applicant notes that the reference furnishes 60 examples; of those 60 examples, the smallest size particle disclosed is found in *prophetic* Example 39A, which discloses a simple albumin-glutaraldehyde mixture that is 200 nanometers in diameter. Besides being at least 4X the size of the claimed nanocapsules, the particles disclosed in this example do not comprise the hydrophobic surfactant or the precipitate shell of the instant claims. Applicant notes the next smallest embodiment in Unger et al's examples is *2.5 micrometers* in diameter (Example 6), which is approximately 3 logs larger than the claimed nanocapsules and 2 logs larger than the particles of Example 39A. Applicant submits these two examples, disclosing those particles with size closest to the claimed nanocapsules, further illustrate the heterogeneity of Unger's disclosures.

With regard to disclosed components and descriptions, the references, including Unger, Medina, and Quay in particular, provide almost limitless materials and limitless vesicle-types, in separate and heterogeneous lists, for formulating the "disclosed" vesicles. For example, vesicles according to Unger comprise "liposomes, micelles, bubbles, microbubbles, microspheres, lipid-, polymer- protein- and/or surfactant-coated bubbles, microbubbles and/or microspheres, microballoons, aerogels, clathrate bound vesicles, and the like" (col. 7, lines 49-55), in which the vesicles may *or may not* include a bioactive agent (col. 60, lines 1-2).

Hundreds of potential surfactants are listed in Quay, beginning at Col. 7, line 15 and continues through Col. 10, line 55. **Unger teaches at least 120 different lipids**, in the section starting Col. 17 and continuing to Col. 21. **Unger teaches the "polymer" for stabilization may include any number of materials, including polysaccharides, cellulosic polymers, polyethylenes, polypropylenes, polyurethanes, polyvinyl chlorides, nylon, polystyrene, synthetic polymers such as polymers of acrylic acid, siloxanes, ethylene glycol, polyethylene glycol**, and so forth (Col. 31 line 1 to line 43.)

Accordingly, then, dictionary-like listings of hundreds of various heterogeneous elements, disclosures covering several orders of magnitude of potential sizes for the liposome, and almost infinite numbers of ways to arrange the elements as taught by the references, it is submitted that in no way can the references be characterized as teaching a "finite number of identified, predictable solutions, with a reasonable expectation of success" toward the nanocapsule system of the instant claims, as required for obviousness under *KSR*. Further, Federal Circuit decisions since *KSR* make it clear that the chemical arts are often "unpredictable." Therefore, considering these *KSR* factors, it is clear that the instant invention is not obvious over the cited references. Reconsideration is respectfully requested.

3. *Examiner relies on impermissible hindsight to combine the elements of Unger in the fashion of the claimed invention*

The Examiner relies upon Unger et al as the primary reference in rejecting the instant claims. Applicant submits Examiner has impermissibly used the claimed invention as a guide to piece together disclosures of Unger et al in an effort to create a mosaic of such disclosures to argue obviousness.

The analysis supporting obviousness should be made explicit. (*KSR International Co. v. Teleflex, Inc.,* 550 U.S. 398, 418 (2007.) According to KSR, "[a] factfinder should be aware, of course, of the distortion caused by hindsight bias and must be cautious of arguments reliant upon ex post reasoning. See *Graham*, 383 U. S., at 36 (warning against a 'temptation to read into the prior art the teachings of the invention in issue' and instructing courts to 'guard against slipping into the use of hindsight' (quoting *Monroe Auto Equipment Co. v. Heckethorn Mfg. & Supply Co.*, 332 F. 2d 406, 412 (CA6 1964.))" *KSR, 550 U.S. 398, 418 (2007.)*

As described above, Unger provides dictionary-like listings of hundreds of various heterogeneous elements, disclosures covering several orders of magnitude of potential sizes for the liposome, and almost infinite numbers of ways to arrange the elements, it is submitted that in no way can the reference be characterized as providing a series of teachings that would lead the skilled person, at the time the invention was made, to the claimed nanocapsules.

Despite the breadth and heterogeneity of Unger's disclosures, the Examiner has not articulated a properly reasoned analysis, from the disclosures of the reference (or the secondary references), to support selecting and combining disparate elements of the primary reference Unger et al in the fashion of the instant invention. It is submitted that Examiner has relied solely upon the Applicant's disclosure, and not the knowledge of an artisan of ordinary skill, to reconstruct the claimed nanocapsules.

KSR states that a reasoned analysis under Section § 103 would include identifying what would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the new invention does. As described above, the Examiner has stated that "improved properties" is the rationale for selecting and combining numerous elements from Unger et al. in the manner of Applicant. However, it is submitted that Examiner has simply generated a list of *individual elements* extracted from Unger et al to match up with elements of the instant claims.

Applicant submits the Examiner has clearly relied upon hindsight and not the understanding of one skilled in the art at the time of the instant invention to *selectively combine* disparate elements disclosed in the primary reference Unger et al, such that the resulting composition, when combined with selected teachings from the secondary references, would have the functional properties required by the instant claims. Examiner has not discharged the initial burden of establishing a *prima facie* case of the subject claims, and withdrawal of these objections therefore is respectfully requested.

4. *The combination of the references does not "teach" all the elements of the instant claims, with particularity to claim 138 and 139.*

Under MPEP 2143.03, all claim limitations must be considered. According to *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974) each claim feature must be present (i.e., taught or suggested) by an asserted combination.

It is submitted that Unger, or any of the other references fail to teach a precipitated shell, particularly, where the cation is Li^+ (see claims 138 and 139.) The Examiner argues, contrary to accepted scientific principles, in the current as well as in previous Actions that "[w]ith respect to the limitation of the protein shell being precipitated by the cation, wherein the cation is Li^+ (claims 66 and 139), this is inherent to the nanocapsules of Unger

et al., since the covalent attachment of the targeting ligand requires addition of lithium aluminum hydride (see above), which would necessarily result in a precipitated protein shell (it is noted that Li^+ is known in the art as a protein precipitating agent, see for example Kondo et al., Abstract)". The Examiner has provided no support for the assertion that the addition of lithium aluminum hydride would "necessarily result" in a precipitated protein shell comprising Li^+.

The Applicant has previously rebutted the Examiner's assertion as factually incorrect (see the Response dated 6/11/08, pages 21-22), and resubmits herein those arguments that the Examiner has not adequately addressed. The instant application teaches a precipitate shell formed using aqueous ions (including, for example, lithium) to displace solute bonding to water (i.e., a phenomenon known as "salting out"). One skilled in the art would understand the resulting precipitate shell would be comprised of, for example, a polypeptide and ions (including for example lithium).

In contrast, Unger teaches the use of the reducing agent lithium aluminum hydride (LAH) to render more permanent, Schiffs-base linkages for covalently coupling ligands to, for example, lipids (a second reducing agent, lithium aluminum diisobutyl hydride (DIBAL), is also listed in Unger, but one skilled in the art would recognize the use of the term "lithium" here to be incorrect, as DIBAL contains no lithium and is thus only an aluminum analog of LAH). This teaching in Unger is *not* equivalent to the claimed lithium precipitate, for several reasons.

First, it is well known in the art that LAH is a highly reactive and nonselective reducing agent such that a conventional practice is to expose the final product (for example the desired ligand conjugate) to LAH for only 30 minutes, in order to degrade it for thin layer chromatography analysis (see Thompson & Lee (1965) BBA 55068:151-9; Wood & Snyder (1967) Lipids 3(20:129-35). LAH reacts explosively with water, and thus conjugations using LAH are executed in *water-insoluble* ether, followed by careful dropwise addition of water to decompose the LAH into its subcomponents (lithium ion, aluminum ion and hydrogen gas), followed by separation of the desired final conjugation product away from the *water-soluble* lithium by multiple extractions with ether (see Nystrom & Brown (1947) *JACS* 69:2548-9; Smith & Ho (1972) *JOC* 37(4):653-6).

Therefore, to avoid degradation of the product conjugate, one skilled in the art would purify the desired product conjugate (e.g., ligand) away from lithium.

Second, it is well established that LAH-mediated reduction reactions are effected by transferring the negative hydride anion, and not the positive lithium cation, to the product (see Nystrom & Brown (1947) *JACS* 69:2548-9; Smith & Ho (1972) *JOC* 37(4):653-6). Thus lithium is a reaction *side product*, not a part of the final product.

Thus, it is clear that the lithium aluminum hydride used to couple ligands as disclosed in Unger is not equivalent, in any way, to cationic lithium used to precipitate the shell in the instant application. Any assertion to the contrary by the Examiner, most particularly regarding the presence of lithium in the final product of an LAH-mediated reduction reaction, is contrary to the scientific principles set forth above. Third, with respect to Kondo, Kondo's taught lithium cation is taught in a process to purify DNA plasmid from cellular RNA and protein. The claimed invention does not teach the use of lithium to remove RNA and protein from DNA. Rather, the claimed invention teaches the use of lithium to stabilize nanocapsules comprising for example DNA cargo *and* a protein shell.

The Applicant has factually demonstrated that the liposome shell of Unger does not inherently contain Li^+ as asserted by the Examiner. MPEP § 2144.03(C) states that when an Applicant adequately traverses a finding not properly based on common knowledge, such as by "specifically point[ing] out the supposed errors in the examiner's action, which would include stating why the noticed fact is not considered to be common knowledge or well-known in the art", then:

> *Zurko*, 258 F.3d at 1386, 59 USPQ2d at 1697 ("[T]he Board [or examiner] must point to some concrete evidence in the record in support of these findings" to satisfy the substantial evidence test). If the examiner is relying on personal knowledge to support the finding of what is known in the art, the examiner must provide an affidavit or declaration setting forth specific factual statements and explanation to support the finding. See 37 CFR 1.104(d)(2)). MPEP § 2144.03(C)

Despite Applicant's previous challenge of Examiner's assertion that the addition of lithium aluminum hydride would "necessarily result" in a precipitated protein shell comprising Li^+, the Examiner has not provided concrete evidence or an affidavit or declaration to support said assertion. Thus, in view of the above, it is submitted that a

legally sufficient "teaching" of the lithium precipitated shell of the inventive particles, was not provided by the Examiner. Reconsideration is respectfully requested.

5. *Long felt need.*

According to MPEP 2141, secondary considerations may rebut any *prima facie* case of obviousness. One such secondary consideration is whether "the claimed invention [satisfies] a long-felt need which was recognized, persistent and not solved by others." Specifically, as reiterated by the Supreme Court in KSR, the framework for the objective analysis for determining obviousness under 35 U.S.C. 103 is stated in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966). Obviousness is a question of law based on underlying factual inquiries. Objective evidence relevant to the issue of obviousness must be evaluated by Office personnel. Such evidence, sometimes referred to as "secondary considerations," may include evidence of commercial success, long-felt but unsolved needs, failure of others, and unexpected results.

It is noted that this section is argued in the alternative, since Applicant does not admit that a *prima facie* case of obviousness is present for the instant claims (see Sections 1-4, above.)

The state of the art at the time viewed targeting vehicle binding to a specific cell receptor for subsequent endosomal uptake into the cell as an attractive drug delivery strategy. Nonetheless, with respect to gene delivery, for example, a contemporaneous review article observes that while this:

> "seemingly simple concept has been pursued for more than a decade…in practice, this idea has been more difficult to implement effectively than perhaps had been originally anticipated….Effective gene delivery by [the] receptor mediated mechanism requires specific vector binding, internalization, subcellular trafficking, endosomal escape, and unpackaging of the foreign DNA for desired gene expression. While this process offers a noninvasive mechanism to obtain selective intracellular localization of vectors, it may lead to destruction of delivered genes through intracellular pathways…"[5]

This review also states that "[e]ndosomal sorting toward the lysosomal degradative fate is thus believed to be the greatest barrier for successful gene expression via non-viral delivery

[5] C.M. Varga, et al. "Receptor Mediated Targeting of Gene Delivery Vectors: Insights from Molecular Mechanisms for Improved Vehicle Design", *Biotechnology and Bioengineering*: 70(6) 593-605 (2000).

vehicles." *Id.* at page 599.

At the time of the instant invention, the most well studied and exploited path for receptor-mediated uptake into a cell was via the clathrin coated-pit path. On the other hand, as described within the instant application, the nanocapsules of the present invention are efficiently taken into the cell via a caveolin-regulated pathway. See for example, Example 2 of the instant specification. With regard to caveolae-based delivery strategies in the art at the time the instant invention was made, a contemporaneous review article observed "there is to date little quantitative evidence showing caveolae to fulfill a role in mediating the uptake of DNA based therapeutics."[6]

Thus, at the time the instant invention was made there had been, for at least ten years, a need in the field of drug delivery to combine cell-targeting and efficient, non-degradative uptake. The emerging view was that any solution was likely to be more complex than originally believed (i.e., unpredictable, with no reasonable expectation of success). Additionally, the art relating to caveolae-based strategies was limited, particularly in comparison to the much-more studied clathrin (degradative) uptake path.

Applicant's nanocapsule system addresses this long felt need, producing cell-targeted, stabilized nanocapsules of sub-50 nm size that avoid substantial lysosomal accumulation and degradation, providing uniform, intact delivery of cargo. See for example, instant application Table 2 (Example 2), which demonstrates that hyaluronan-coated nanocapsules substantially avoid lysosome co-localization, while PEI-DNA and lipoplex-plasmid DNA complexes did not. See also for example Figure 7 (Example 6), which demonstrates that tenascin-coated nanocapsules bearing plasmid DNA provided targeted, uniform, high-intenstity GFP expression in tumor cells, reflecting successful intact delivery to the nucleus.

Heidel et al. summarized the views held by many for years, i.e., that addressing the challenges of drug delivery isn't simply a matter of identifying useful ingredients or adding components to known vesicle structures; "the challenges of creating a nonviral delivery system are many[, but] we believe that one of the most daunting….is the integration of the components into a workable system that combines the attributes of the components without

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[6]M. Gumbleton, et al. "Caveolae: An Alternative Membrane Transport Compartment", *Pharmaceutical Research:* 17(9) 1035-1048 (2000).

suffering losses because of their integration."[7] This was exemplified for example in Kong et al., which reported that in a study of tumor-specific liposomes of a range of sizes, "[l]iposomes < 100 nm in diameter were not made because of instability." G. Kong et al., Hypothermia Enables Tumor-specific Nanoparticle Delivery: Effect of Particle Size, *Cancer Research*: 60, 4440-4445 (2000). In another example described further below in this response, Schneider et al found that when polypeptide-DNA complexes were associated with a delivery vehicle (LipofectAMINE™), targeting specificity was reduced, leading to the conclusion that "in the presence of the LipofectAMINE™ internalization of the complex occurs to a large extent by an integrin-independent mechanism."

Accordingly, based on the objective evidence that (1) delivery strategies involving vehicle binding to targeted cell receptors for subsequent endosomal uptake and intact delivery were difficult to achieve, and (2) integration of nanoparticle components into a "workable" system has challenged practitioners, it is submitted that the claimed nanocapsules meet significant, long felt needs and are a significant leap over the teachings of the prior art. It is therefore respectfully submitted that Applicant's demonstration of meeting a long-felt need is further evidence of nonobviousness, and it is requested that this evidence be considered by the Examiner.

B. Rejection of claims 66, 67, 87, 87-94, and 133-141

The Examiner has also rejected claims 66, 67, 87, 87-94, and 133-141, under 35 U.S.C. § 103(a) as being unpatentable over Unger, E.C. et al. (U.S. Patent No. 6,139,819, the "Unger et al" patent) in view of each Kondo et al., in view of each Medina (U.S. Patent No. 5,650,543), Quay (U.S. Patent No. 5,707,606) and Duquemin et al. (J Pharm Pharmacol, 1985, 37:698-702, Abstract), further in view of Schneider et al. (FEBS Letters 1998 429:269-273).

The Examiner references the rejections made above for claims 66, 67, 87, 88, 90-94, and 135-139, and notes that these references do not teach tenascin. The Examiner contends that Schneider teaches identification of a polypeptide derived from the C-terminus of tenascin, and also a ligand that targets a receptor for tenascin. Schneider also teach their

[7] J. Heidel et al., "Molecular Conjugates", *Adv. Biochem. Engin./Biotechnol*: 99, 7-39 (2005). Copy provided in IDS accompanying this response.

peptide as being suitable to mediate specific gene delivery to $\alpha_9\beta_1$ integrin-expressing cells. The Examiner contends that the motivation to modify the references applied above with Schneider et al.'s teachings for the reason that Schneider teaches that targeting $\alpha_9\beta_1$ integrin is promising for gene therapy delivery vehicles, and Unger et al. teach that peptide ligands can be successfully included in their nanocapsules.

Applicant traverses this rejection. Applicant incorporates by reference the arguments made above in relation to the rejection of claims 66, 67, 87, 88, 90-94, and 135-139. It is submitted that the rejection fails for the reasons identified above, and Schneider et al. fail to remedy the deficiencies of the references.

Applicant submits that the Schneider provides conflicting teachings with regard to tenascin. In another Schneider et al. journal article, published prior to the filing of the instant invention, (Schneider et al., FEBS Letters, 1999, 458:329-332, copy provided herein), the authors investigated the ability of the polypeptide to mediate specific gene delivery.[8] They found that when polypeptide-DNA complexes were associated with the liposome "LipofectAMINE^{TM}", targeting specificity was reduced, leading to the conclusion that "in the presence of the LipofectAMINE^{TM} internalization of the complex occurs to a large extent by an integrin-independent mechanism." (p. 331)

Applicant submits that one skilled in the art, upon reading the further work of Schneider et al., would understand that the studied polypeptide was not effective in targeted gene delivery when associated with a delivery vehicle, and would consequently view this art as teaching away from the instant invention. "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, … would be led in a direction divergent from the path that was taken by the applicant." *Tec Air, Inc. v. Denso Mfg. Mich. Inc.*, 192 F.3d 1353, 1360, 52 USPQ2d 1294, 1298 (Fed. Cir. 1999). Applicant therefore submits Schneider et al either provides neither motivation nor a basis for a reasonable expectation of success to modify the vesicles of Unger et al and secondary references, with regard to incorporating the ligand described in the Schneider et al reference and article.

Reconsideration is respectfully requested.

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[8] The 1998 article reported on studies of the naked, linear polypeptide PLAIEDGIELTY; the 1999 article reported on PLAIEDGIELTY as well as its cyclic version, GCPLAEIDGIELCA. When the peptides were covalently bound with DNA and complexed with LipofectAMINE, results were similar for both.

For the reasons set forth above, Applicant respectfully submits the claims as filed are allowable over the art of record and reconsideration and issuance of a notice of allowance are respectfully requested. If it would be helpful to obtain favorable consideration of this case, the Examiner is encouraged to call and discuss this case with the undersigned.

This constitutes a request for a three-month extension of time pursuant to 37 C.F.R. § 1.136(a) and an authorization to charge all fees therefor to deposit account No. 19-5117. The undersigned hereby authorizes the charge of any fees created by the filing of this document or any deficiency of fees submitted herewith to deposit account No. 19-5117.

Respectfully submitted,

Date: March 15, 2010

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